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(21) International Application Number: PCT/EP99/04035 (22) International Filing Date: 8 June 1999 (08.06.99) (30) Priority Data: S980434 8 June 1998 (08.06.98) IE (71) Applicant (for all designated States except US): GLENCAS- TLE ENTERPRISES LTD. [GB/GB]; Exchange House, 4th floor, 54-58 Athol Street, Douglas, Isle of Man IM1 1JD (GB). (72) Inventor; and (75) Inventor/Applicant (for US only): LYNCH, Edward [IE/GB]; 90 South Croxted Road, London SE21 8BD (GB). (74) Agent: DUFFY, Assumpta; F.R. Kelly & Co., 27 Clyde Road, Ballsbridge, Dublin 4 (IE).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the</i> <i>claims and to be republished in the event of the receipt of</i> <i>amendments.</i>
(54) Title: USE OF OZONE FOR THE PREPARATION OF MEDICAMENTS FOR THE TREATMENT OF DENTAL CARIES		
(57) Abstract This invention concerns the use of ozone in the treatment of dental caries.		

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USE OF OZONE FOR THE PREPARATION OF MEDICAMENTS FOR THE TREATMENT OF DENTAL CARIES

This invention relates to the use of ozone in the treatment of dental caries.

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The great destructive disease of teeth is dental caries which may be defined as the acid dissolution of enamel, dentine or cementum as a consequence of the metabolism of micro-organisms living within deposits on the teeth
10 known as plaque. Dental caries is believed to be associated with specific micro-organisms, the principal ones being *Streptococcus Mutans*, *Lactobacilli*, *Actinomyces Visosus Sero var 2*, *Actinomyces Naeslundii* and "Intermediate" *Actinomyces*, other *Streptococci* and
15 yeasts. These are acid producing micro-organisms which produce acids such as acetic and lactic acids from the dietary carbohydrates. The micro-organisms associated with dental caries are unique and are ecologically very different from those associated with, for example,
20 infected root canals.

Dental caries is currently managed by one or more of the following:

- 25 (i) preventive treatment by, for example, dietary and oral hygiene measures and may include the topical application of chemotherapeutic agents;

(ii) the removal of dentine exhibiting the signs of active caries;

(iii) the protection of any newly exposed non-carious dentine with restorative material.

5

Measures aimed at the prevention or the arrest of dental caries are mainly based on the elimination of dental plaque from the surfaces of roots and the
10 institution of dietary controls to reduce the frequency and quantity of readily fermentable carbohydrate ingestion. The mechanical removal of plaque has been a major platform for the prevention of dental caries for some time. However, this poses special problems in the
15 case of primary root caries due to access problems. Because dentine has a Knoop hardness of 68 in contrast to enamel at 11, the mechanical removal of plaque from its surface inevitably results in some loss of tissue also. Toothbrush abrasion is now a very common
20 phenomenon and invariably leads to the loss of root dentine from the facial aspects of teeth. Consequently, the traditional methods of plaque control in the prevention of dental caries create further problems even when access permits it to be used
25 effectively.

Conventional caries removal and cavity preparation entail the use of high and low speed handpieces. However, disadvantages of this system include the

perception that drilling is unpleasant for patients and local anaesthetic is frequently required. Furthermore, handpieces are expensive to purchase and maintain and their use may lead to the removal of softened but
5 uninfected dentine resulting in the excessive loss of tooth tissue.

Where restoration is required, all materials used to restore carious lesions have their limitations. For
10 example, gold and ceramic are expensive and present a technical challenge for the practitioner. While amalgam is a durable, predictable material, it has poor aesthetic qualities, is potentially toxic and may cause allergic reactions in some people.

15

It is an object of the invention to alleviate the disadvantages of the prior art.

It has now unexpectedly been found that ozone can
20 penetrate carious tissue and can therefore be used in the treatment of dental caries.

According to the present invention there is provided the use of ozone in the preparation of a therapeutic
25 system for the treatment of dental caries.

As used herein, the term "ozone" is intended to embrace pure ozone, oxonised air and ozonised aqueous media,

such as water optionally containing a reductant, such as thiocyanate or peppermint.

The ozone is delivered at a pressure sufficient to
5 penetrate the carious tissue and at a concentration and
for a period of time sufficient to kill substantially
all of the micro-organisms within the carious lesion.

Preferably, a needle-sized jet of pure ozone or
10 ozonised air in a shroud of micro-organism-free aqueous
medium, e.g. water optionally containing a reductant,
is injected at the desired location.

If desired, a sealant of the type known in the art may
15 be applied to a carious lesion following ozone
treatment.

The advantages of using ozone in the treatment of
dental caries include the following:
20

1. It eliminates drilling and its attendant problems;
2. It is rapid and painless;
- 25 3. It does not require sophisticated methods of
isolating the tooth;
4. No local anaesthetic is required.

The invention is illustrated in the following Examples. Unless otherwise stated, the ozone delivered in the following Examples is present in air at a concentration of 5.2%.

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Example 1

Many studies concerning the clinical evaluation of ozone have been based on assessments of its harmful effects rather than demonstrating any therapeutic benefits it may offer. Ozone is one of nature's most powerful oxidants which accounts for its ability to kill bacteria, spores and viruses. Uniquely, ozone decomposes to a harmless, non-toxic and environmentally safe material (oxygen). In this investigation, a multicomponent evaluation of the oxidative consumption of salivary biomolecules by ozone (O_3) has been performed using high resolution proton (1H) nuclear magnetic resonance (NMR) spectroscopy. The ozone-generating equipment employed in this study was designed by Purezone Ltd. (Ipswich, U.K.).

Unstimulated human saliva samples were collected from 8 patients and each of them was divided into two equivalent portions (0.60ml). The first of these was treated with O_3 generated from the above device for a period of 30 seconds; the second group of portions served as controls. Samples were subjected to 1H NMR analysis at an operating frequency of 600 MHz. Results acquired revealed that O_3 treatment gave rise to (1)

the oxidative decarboxylation of the salivary electron-donor pyruvate (generating acetate and CO₂ as products), (2) oxidation of the volatile sulphur compound precursor methionine to its corresponding sulphoxide and (3) the oxidative consumption of salivary polyunsaturated fatty acids. Moreover, evidence for the O₃-mediated oxidation of salivary 3-D-hydroxybutyrate was also obtained. High field ¹H NMR spectroscopy provides much useful analytical data regarding the fate of O₃ in human saliva, information which is of much relevance to its potential therapeutic actions in vivo.

Example 2

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Ozone Effect on Microflora from Primary Root Caries Ex-vivo

Primary root carious lesions (PRCL) are a major clinical problem. The aim of this study was to establish if ozone could achieve effective microbial killing in PRCL. An ozone producing generator (Purezone Ltd., Ipswich, U.K.) was used in this ex-vivo study assessing the use of ozone on PRCL. In this study, soft PRCL requiring restoration were used as these are the most severe type of lesion found in humans. 20 freshly extracted teeth with PRCL requiring restoration were used. After plaque removal using a hand held standard fine nylon fibre sterile toothbrush

with sterile water as a lubricant to cleanse the surface, each tooth was then isolated using sterile cotton wool rolls and dried using a dry sterile cotton wool roll. A sample of PRCL was taken using a sterile excavator from half of the most active part of the lesion. Subsequently, 10 seconds of the ozonised water was applied to the lesion and another sample was taken from the other half of the most active part of the lesion. Each sample was weighed and immediately placed in 1 ml of Fastidious Anaerobe Broth (FAB). To each 1 ml of FAB containing a biopsy of carious or ozone treated carious dentine, sterile glass beads were added. They were vortexed for 30 seconds to facilitate the extraction of any micro-organisms from the carious dentine and disperse any aggregates. After decimal dilution with FAB, 100 ml aliquots of these was spread on Fastidious Anaerobe Agar (LabM, Bury, Lancs., U.K.) supplemented with 5% (V/V) horse blood in an anaerobic chamber at 37°C for four days. The mean \pm SE number of each colony type was counted and calculated.

	Before Ozone Treatment	After 10 Seconds of Ozone Treatment
Mean \pm SE of		
total cfu (Log_{10})	5.91 \pm 0.15	3.57 \pm 0.37

Using the paired Student t-test a significant difference ($p < 0.001$) was observed between the two groups. Clearly, the percentage of micro-organisms

killed associated with the use of ozone was more than 99%.

Example 3

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Ozone Effect on Microflora from Primary Root Caries *Ex-vivo*

The procedure of Example 2 was repeated except that
ozonised water was applied to the lesion for 20
10 seconds. Using the paired student t-test, a
significant difference was observed in the ozonised
water group ($\log_{10} 3.77 \pm 0.42$, mean \pm SE) compared
with the control group ($\log_{10} 6.18 \pm 0.21$) ($p < 0.001$).

The results of these tests show that the use of ozone
15 can provide an effective, rapid and simple means for
killing micro-organisms in carious lesions.

Example 4

Sealant Shear Bond Strength to Sound and Carious 20 Radicular Dentine

There has been little research on the interaction
between primary root carious lesions (PRCL) and
adhesive materials. The aim of this study was to
examine the shear bond strength of four adhesive
25 systems to PRCL with sound dentine acting as a control.
The adhesive systems used were:

1. OptiBond FL Prime¹/OptiBond FL Adhesive¹/OptiGuard¹
2. OptiBond FL Prime/OptiGuard
3. OptiGuard and
- 5 4. ChemFil II²

The materials were applied to sound radicular dentine and PRCL *in vitro* in freshly extracted teeth. The bonding site was macroscopically intact, was flat and had at least a 3.5 mm diameter. 37% phosphoric acid
 10 was used for 15 seconds in samples in groups 1→3 whilst 25% polyacrylic acid was used in group 4. After bonding the samples were stored for seven days in a moist atmosphere at 37°C. A shearing force was applied at 1 mm/minute. There were at least 10 samples in each
 15 group. The mean (s.e.) shear bond strengths were (MPa);

Adhesive	Control	Carious
OptiBond FL Prime/OptiBond FL Adhesive/OptiGuard	5.31 (1.03)	5.58 (1.05)
OptiBond FL Prime/OptiGuard	2.01 (0.59)	1.63 (0.40)
OptiGuard	0.73 (0.24)	1.45 (0.52)
ChemFil II	1.42 (0.28)	1.01 (0.26)

While statistical testing showed that the shear bond strength of the OptiBond FL Prime/OptiBond FL Adhesive/OptiGuard was significantly the highest, (p<0.001), the caries status of the root surface had no significant influence on the bond strength. OptiGuard in combination with OptiBond FL Prime and OptiBond Adhesive had the highest bond strength and this was not influenced by the caries status of the surface.

¹Kerr, Romulus, Michigan, U.S.A.;

²Dentsply, Konstanz, Germany.

Example 5

15 The effect of ozone on primary root caries and associated micro-organisms

The aims of these studies were to evaluate the efficiency of ozone on primary root caries and associated micro-organisms (*Streptococcus sobrinus*; TH 21, *Streptococcus mutans*; NCTC 10449). In study 1, 40 soft primary root carious lesions (PRCLs) from freshly extracted teeth were used and randomly divided into two groups to test the exposure to ozone for either 10 or 20 seconds. There was a significant (p<0.001) difference (Mean \pm SE) between the control samples for.

either 10 seconds ($\log_{10} 5.91 \pm 0.15$) or 20 seconds ($\log_{10} 6.18 \pm 0.21$) and ozone treated samples for either 10 seconds ($\log_{10} 3.57 \pm 0.37$) or 20 seconds ($\log_{10} 3.77 \pm 0.42$). In study 2, 40 sterile saliva
5 coated glass beads were put into bijoux bottles with 3 mls of Todd Hewitt broth for control and test groups. *S. sobrinus* and *S. mutans* were inoculated and incubated anaerobically overnight. Each glass bead was washed with 2 mls of PBS. Immediately, 10 seconds of ozone
10 was applied to the glass beads in the test groups. Subsequently, each glass bead in the test and control groups was placed in 3 mls of Todd Hewitt Broth with six more sterile glass beads and were vortexed for 30 seconds. After decimal dilutions, 100 ml aliquots were
15 spread on blood agar plates supplemented with 5% (V/V) horse blood and placed in an anaerobic chamber at 37°C for two days. The number of each colony type was counted and calculated. Using the paired student t-test, there was a significant reduction ($p < 0.0001$)
20 (Mean \pm SE) between the control samples for *S. sobrinus* ($\log_{10} 4.61 \pm 0.13$) and *S. mutans* ($\log_{10} 3.93 \pm 0.07$) and ozone treated samples for *S. sobrinus* ($\log_{10} 1.09 \pm 0.36$) and *S. mutans* ($\log_{10} 1.01 \pm 0.27$). This treatment regime is therefore an effective, quick,
25 conservative and simple method to kill micro-organisms in primary root carious lesions.

CLAIMS:

1. Use of ozone in the preparation of a medicament for the treatment of dental caries.

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2. Use according to claim 1 wherein the ozone is delivered for at least 0.5 second.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/E. 99/04035

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K33/44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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A	DATABASE WPI Section PQ, Week 199711 Derwent Publications Ltd., London, GB; Class P32, AN 1997-113099 XP002122889 & JP 09 000548 A (EXCELLENT YG), 7 January 1997 (1997-01-07) abstract --- -/--	1,2

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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